NOTES ON THE ANATOMY OF THE TREMATODE, MICROPHALLUS OPACUS.*

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This investigation has been carried on with material furnished by Dr. H. B. Ward from type specimens of *Microphallus opacus* collected in 1893 from *Amia calva* in Lake St. Clair. I was unable to use whole mounts to any great extent. Most of the work was done with transverse, frontal and sagittal sections, 5-20μ thick and stained on the slide with Ehrlich's haematoxylin. This species was first described by Ward in 1894 as *Distoma opacum*. Its present genus, *Microphallus*, of which it is the only member was founded by Ward in 1901. A description of the general appearance and of the suckers may be found in Ward (1894) and need not be repeated.

The body wall is of the usual type among trematodes. There is a noncellular cuticula, several layers of muscle fibres and a region of closely packed cells merging with the looser parenchyma. The cuticula presents a finely dotted appearance, the nature of which was not determined. Adjacent to the cuticula is a layer of very fine muscle fibres, a little over 1μ apart. Next is a layer of coarse longitudinal fibres and two layers of coarse oblique fibres making angles of 60° with each other and the longitudinal, the three thus forming a pattern of equilateral triangles. The fibres in these layers are at varying distances apart depending upon the part of the body examined. They are most abundant in the anterior ventral region. The cuticula continues into the digestive tract lining the prepharynx and esophagus. Coarse longitudinal fibres, some two dozen in number, lie below the cuticula in these regions. The pharynx is composed mainly of radial muscle fibres but has a layer of circular fibres on the inside and anteriorly on the outside.

The excretory system consists of sixteen large flame cells, a system of canals, a large bilobed vesicle, and a short ciliated canal

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to the exterior (Fig. 2). The details were obtained by reconstructing the system in four series of transverse sections. The reconstructions were made by measurements with the ocular micrometer and plotted on a previously reconstructed lateral or dorsal view of the animal. These four series checked with each other. The number and position of the flame cells was checked in several other series. The excretory pore as a rule is placed dorsally and slightly in front of the extreme end. It is at the end of a short, thickly ciliated canal, the outlet of a vesicle which extends forward for one-third to two-fifths of the length of the body. Almost from the first the vesicle shows median longitudinal constrictions on the dorsal and ventral surfaces, and at one-third to one-half its length it divides completely into right and left lobes. These are more or less cylindrical, tapering somewhat at the anterior end. The whole vesicle lies fairly close to the dorsal surface. The lining of the vesicle and its outlet are crowded with nuclei but muscle fibres were not detected.

The system of canals and flame cells is bilaterally symmetrical, eight flame cells on each side of the body. There is also a certain biradial symmetry. As mentioned above, the vesicle lies along the dorsal surface of the body. A canal drops to the ventral surface from the anterior end of each lobe and divides into a posterior and an anterior branch. Four flame cells supply the posterior and four the anterior and with corresponding canals. In fact, all of the flame cells are similarly placed with respect to canals; all have just three forks between them and the excretory vesicle. In each of the pairs of flame cells which are immediately connected by a fork, one is decidedly dorsal, the other ventral.

The details of the system can best be described by considering one side of the body only (Figs. 1 and 2). The first ventral flame cell is at the side of the anterior sucker, often closely against it. The first dorsal is above the esophagus, a little off the median line. Canals run from them and unite near the side of the body. The united canal runs back longitudinally. The second ventral flame cell is lateral to the intestinal branches or seminal vesicle and not far from the ventral surface. The second dorsal is above these organs and near the median line. In some specimens the second ventral or second dorsal flame cell is close against the seminal vesicle or ovary,

but this seems to be an accidental relation. The canals of this pair unite a little above the longitudinal canal from the first pair of flame cells and are joined with the latter by a very short canal. In one case this short canal was shortened to disappearance on one side. Beyond the junction the canal continues longitudinally to the region along side of the ventral sucker where it is joined by the corresponding canal supplied by the third and fourth pairs of flame cells. The fourth ventral flame cell is near the posterior end of the animal, pressed back by the last fold of the uterus. The fourth dorsal is close against the posterior side of the testis on that side of the body. Canals from these two run toward each other and toward the side of the body all the time confined in the narrow space between uterus and body wall. They are usually pressed flat and difficult to follow. The united canal runs forward and downward still outside the uterine folds. The third ventral flame cell is near the side of the ventral sucker. The third dorsal is close against the anterior face of the testis. I found one exception in which it was considerably anterior to the testis. The canals of this pair run outside the folds of the uterus and join near the ventral surface. The resulting canal runs posteriad to a union with the canal supplied by the fourth pair. The canal thus formed runs in toward the ventral sucker to join the canal supplied by the first four flame cells on that side of the body as mentioned above. The large canal following this union runs perpendicularly upward between the folds of the uterus, the only one to do so, to the anterior end of the lobe of the excretory vesicle on its side.

These flame cells are very favorable for study. They are very large and of typical form, each cell having a conspicuous nucleus, cytoplasmic processses in all directions, and at one side a conical bunch of cilia some 15μ long which projects into the blind end of a canal.

The great simplicity of the excretory system may be merely correlated with the small size of the animal and due to degeneration, but it seems not unlikely that it has more significance. In the fact that the main trunks divide in the middle of the body into anterior and posterior branches, it is a good representative of Looss' typical form for distomes of which he (1894) considers such forms as that in which the main trunks go to the anterior end of the body and re-

turn on themselves, or in which the system is a complex network, to be later modifications.

In the nervous system, the large nerves show fairly well on slides stained with haematoxylin as gray bundles of fibres in a blue background. The smaller nerves, I frequently had trouble in following from section to section. Only the large nerves could be followed in transverse sections. Various tangential sections gave the best results.

The central part of the nervous system is a large mass forming an arch over the prepharynx just in front of the pharynx (Fig. 3). Radiating from each end are nerves connecting with the eight longitudinal trunks an dnerves supplying the oral sucker. Six nerves are given off at each end, four in a more or less horizontal plane (Fig. 4), one upwards and one downwards. The largest one runs posteriad from the central mass and lateriad at an angle of about 30°. Near the end of the first third of the body it drops down to the ventral surface and continues back along this surface curving inward somewhat in the region of the ventral sucker. This is the main trunk. The next nerve in order in the horizontal plane is a very fine one, at right angles to the long axis of the body and connecting with the lateral trunk. Next comes a large bundle going forward and out some 45° and dividing into a short branch running to the sides of the oral sucker and the large lateral trunk which runs far back along the lateral margin of the body. A large but short branch runs directly forward over the dorsal surface of the oral sucker. The ventral bundle runs outwards and forward somewhat and is connected at the ventral surface with the corresponding nerve on the other side by a commissure. Two small nerves run forward from points near the connection and the fairly large ventral longitudinal trunk runs backward one-fourth to one-third the length of the animal. It stops just where the main trunk reaches the ventral surface. The dorsal bundle from the central mass divides at the dorsal surface, the forward branch running to the anterior end of the body, curving down with the curve of the body and ending just above the oral sucker while the backward branch is the dorsal longitudinal trunk and runs back parallel to the long axis of the animal. A short distance back of the central mass the main trunk sends out large nerges to the ventral, lateral, and dorsal trunks (Fig.

5). The dorsal and lateral trunks and the nerves on the ventral surface, the ventral and main trunks, are connected by commissures. In my preparations I could not determine the number satisfactorily and in the case of those connecting with the lateral trunks none were found which could certainly be demonstrated as complete commissures. The main trunk sends a couple of branches from each side to the ventral sucker. These seem to run directly into the circular muscles surrounding the base of the sucker. The system in the posterior part of the body could not be determined at all.

This arrangement of the nervous system agrees in most respects with that which I have found described for other distomes. I have not, however, found reference to the ventral longitudinal trunk. The main trunk is the one usually called ventral. As the ventral trunk is immediately below the main trunk, close to it, and smaller, it would probably be difficult to distinguish in whole mounts.

Only a few additions need be made to the description of the reproductive system by Ward (1894 and 1901). There is a curious interweaving of the vasa deferentia, the yolk ducts and the uterus (Figs. 8 and 9). The vasa deferentia run forward and downward from the testes and unite in the middle region of the body. The yolk ducts run forward and upward from the yolk glands and unite above the union of the vasa deferentia. Instead however of passing each other symmetrically, the right yolk duct passes in front of below the right vas deferens while the left yolk duct passes over and behind the left vas deferens. The uterus passes in front of the yolk duct but behind the vasa deferentia. The yolk ducts are distorted from bilateral symmetry somewhat, the vasa deferentia more. This was the relation in all series examined including one described later in which there were two ovaries so that the reproductive organs were unusually symmetrical.

Ward (1894) after stating that as the animals become mature the yolk glands increase in size, continues thus: "In older specimens, I am inclined to think that they are again reduced in size but evidence on this point is incomplete." My slides are not of the right stages to give evidence on an early increase in size, but they clearly show a later reduction. The changes in the size of all the reproductive organs are very interesting. In young specimens the yolk ducts end in large masses occupying most of the sides of the posterior half

of the body. Each gland is composed of a number of large spherical lobes, some fused side by side and some connected by small ducts in an irregular way. In these specimens the uterus is a narrow tube winding around in the parenchyma between the two yolk glands; the ovary and testes are large and round often over one-half the thickness of a cross section, while the seminal vesicle may be rather small, often smaller than the ovary (Fig. 6). On the other hand, in older specimens the lobes of the yolk glands lose their spherical appearance and become irregular masses compressed into the mould of the uterine folds which here fill the hind body so completely that almost no parenchyma can be seen (Fig. 7). In some cases nothing can be found of the yolk glands but the stumps of their ducts and perhaps a few scattered yolk cells. Here the testes are smaller and compressed into an irregular shape, and the ovary is smaller, while the seminal vesicle becomes larger than the ovary, sometimes swollen to reach almost from the dorsal to the ventral surface. Thus the organs which produce the components of the egg—ova, sperm, and shell, reach their maximum early. The organs which store the sperm and eggs, reach their maximum later at the expense of the former set. The changes in the yolk glands and uterus are most striking.

At first sight the uterus seems to fill the hind body with a series of irregular, chance folds. These folds cannot be followed in whole mounts owing to concealment by the yolk glands or to their own complexity. Examination of sections shows, however, that there is a simple and fairly constant plan beneath the secondary complexities. I followed the convolutions in nine series. Seven followed the plan below perfectly, the two others differed slightly. The method of study was the reconstruction of serial sections, transverse in eight cases, frontal in the other. The general plan (Figs. 10 and 11) is this: The uterus drops down from the shell gland and enlarges in the center of a cross section in the middle region of the body, proceeds posteriad, enlarging all the time, finally loops forward on the right side, dorsal to the yolk glands, and reaches its starting point. Then it doubles back on itself, retracing its course but ventral to the yolk glands and again reaches the starting point. Then it forms the same folds on the left side but in reverse order and the final portion, become small again, goes forward side by side with

the first portion and where the latter rises toward the shell gland, the former drops to leave the body at the genital pore. The dorsal circuit on both sides is typically quadrilateral in shape tho there are usually additional minor twists in it. The lateral and anterior sides are the most enlarged portions of the uterus. Sometimes they have a diameter one-fourth that of the body. The ventral circuit is typically triangular, in fact almost invariably has only two bends.

One very remarkable specimen was examined (Fig. 9). Aside from minor peculiarities it was distinguished by having two ovaries. This specimen was the shortest in proportion to its other dimensions of any examined. The dimensions were 1.0x0.7x0.55 mm., length, breadth and thickness. The anterior sucker and digestive tract were normal except that the branches of the intestine were unusually short and diverged at right angles to the esophagus, being closely pressed against the seminal vesicle. The ventral sucker was extremely far back. The opening was at the beginning of the last third of the body. Its breadth was about normal but it was unusually low and very long, far from the normal spherical shape. Its deep cavity was turned posteriad. The seminal vesicle was centrally placed and globular. The ductus ejaculatorius, instead of dropping straight downward, went almost directly posteriad and with the metraterm left the body at an extremely oblique angle. The opening was, however, left of the ventral sucker as is normal. Transverse sections normally split the ductus; here they showed a nearly circular cross section. The two ovaries were exactly paired with each other, right and left. Both were dorsal to the seminal vesicle. Both were ellipsoidal and closely followed by a normal testis. The left ovary, the abnormal one, was considerably the larger. It had no oviduct. The outlet of the right was normal tho somewhat displaced in parts. There was more symmetry in the position of Laurer's canal, the first part of the uterus and the yolk ducts than usual. The relations of the yolk ducts and vasa deferentia were however asymmetrical as mentioned before.

Summary

The principal points discussed are:

(1) The nature of the integument.

(2) The excretory system, particularly its biradial symmetry and the similar relations of all the sixteen flame cells in the system.

(3) The nervous system, particularly the presence of eight longitudinal nerves.

(4) The asymmetrical relation of the yolk ducts and vasa deferentia.

(5) The alternation in maximum development of yolk glands, ovary and testes on one hand with seminal vesicle and uterus on the other.

(6) The arrangement of the folds of the uterus.

(7) A specimen with two ovaries.

In conclusion I wish to express my gratitude to Professor Henry B. Ward for the material and for the aid and encouragement he has given me in this work.

Papers Cited

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Explanation of Plates

Abbreviations

CNM Central nerve mass
DE Ductus ejaculatorius
DLN Dorsal longitudinal nerve
EC Excretory canal
EV Excretory vesicle
FC Flame cell
Int Intestinal branch
LC Laurer's canal
LLN Lateral longitudinal nerve
M Metraterm
MLN Main longitudinal nerve
Oes Esophagus
Os Oral sucker
Od Oviduct
Ov Ovary
P Copulatory papilla
Ph Pharynx
Pre Prepharynx
SG So-called Shell gland
SV Seminal vesicle
T Testis
Ut Uterus
VS Ventral sucker
YD So-called Yolk duct
YG So-called Yolk gland
1D First dorsal flame cell
1V First ventral flame cell
2D etc. Second dorsal flame cell, etc.

Plate XVII

Fig. 1. Excretory system of left side, in lateral aspect, reconstructed by measurements from transverse sections. Somewhat foreshortened. ×60.

Fig. 2. Excretory system. Dorsal view. Reconstructed from same series as Fig. 1. ×60.

Fig. 3. Nervous system. Dorsal view. Diagramatic. ×60.

Fig. 4. Frontal section, showing the nerves leading from the central nerve mass at one side. ×75.

Fig. 5. Transverse section showing the eight longitudinal nerve trunks and two commissures. ×75.

Plate XVIII

Fig. 6. Transverse section thru posterior part of body, showing well developed yolk glands and small uterus. ×45.

Fig. 7. Transverse section similar to that of Fig. 6 but in another series, showing remnants of yolk glands and enlarge uterus. ×45.

Fig. 8. Reconstruction from several transverse sections, showing typical arrangement of reproductive organs (except middle of uterus) from posterior end. ×75.

Fig. 9. Similar reconstruction to Fig. 8. Made from the specimen with two ovaries. ×75.

Fig. 10. Ventral view of the folds of the uterus, reconstructed by measurements from transverse sections. ×60.

Fig. 11. Similar view to Fig. 10. Showing a younger stage. ×60.

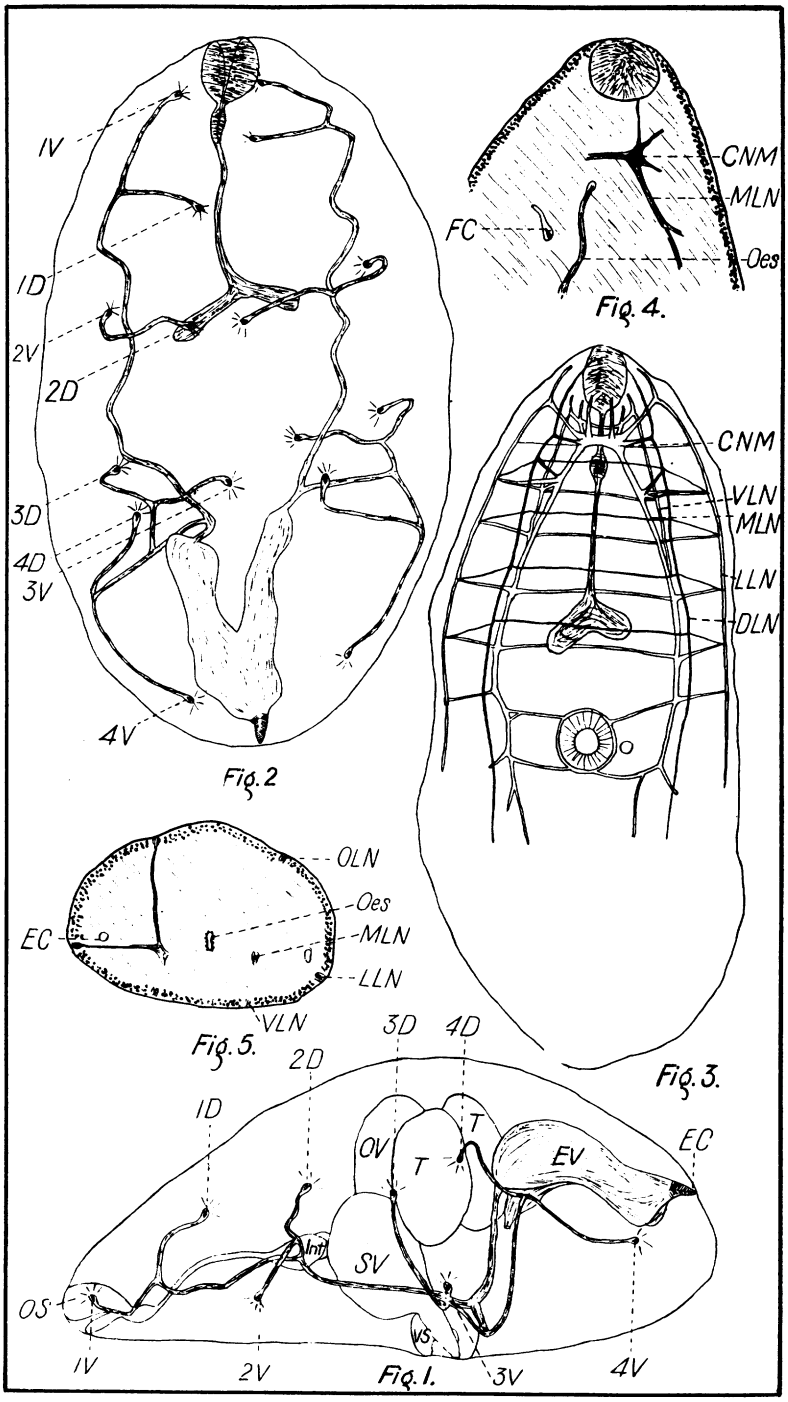
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Fig. 2
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Fig. 4.
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VLN
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Fig. 3.
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Fig. 5.
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Fig. 1.
3V
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PLATE XVII

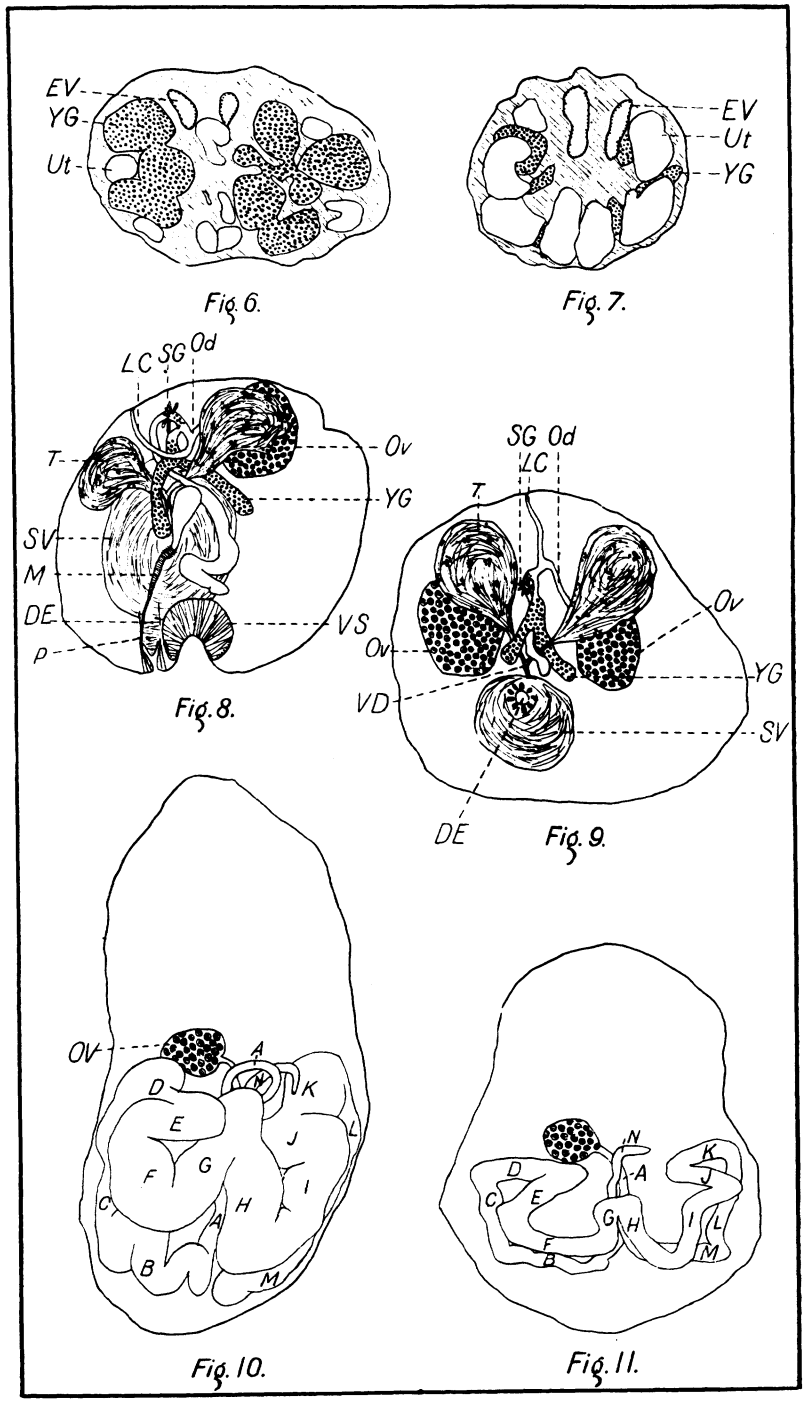
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Fig. 10.
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Fig. 11.

PLATE XVIII